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CHANGES IN LIVER PARENCHYMA OF GREEN FROGS (*PELOPHYLAX ESCULENTUS* COMPLEX) UNDER CONDITIONS OF ANTHROPOGENIC POLLUTION AND THEIR USE IN MONITORING OF WATER BODIES

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Changes in Liver Parenchyma of Green Frogs (*Pelophylax esculentus* complex) under Conditions of Anthropogenic Pollution and Their Use in Monitoring of Water Bodies. Akulenko, N. M. — Determination of the total biological effects of anthropogenic water pollution remains an important issue. Our long-term studies have shown that numerous alterations occur in the frog liver histological structure under pressure of anthropogenic pollution factor. The leukocyte infiltration, fat dystrophy, protein dystrophy and necrosis are well known on the mammal liver. Also we first described the rebuilding in the normal liver structure and the depletation of the fibrous tissue which are characteristic for the amphibians. Quantitative analysis of these alterations can identify significant differences in the pattern of pathological changes in the liver of the green frogs, which pick up in anthropogenically contaminated landscapes and in the clear ponds. This method allows quantifying the degree of biological effect of pollution.

Key words: amphibia, liver, anthropogenic pollution, monitoring.

Изменения в паренхиме печени зеленых лягушек (*Pelophylax esculentus* complex) в условиях антропогенного загрязнения и их использование для мониторинга водных объектов. Акуленко Н. М. — Определение совокупного биологического воздействия антропогенного загрязнения воды остается важным вопросом. Наши долгосрочные исследования показали, что в печени лягушки под действием антропогенного загрязнения происходят многочисленные изменения гистологическогой структуры. Инфильтрация лейкоцитов, жировая дистрофия, белковая дистрофия и некроз хорошо известны для печени млекопитающих. Также мы впервые описали перестройки нормальной структуры печени и уменьшение количества фиброзной ткани, которые характерны для земноводных. Количественный анализ этих изменений выявляет достоверные различия в количестве патологических изменений в печени зеленых лягушек, которые забраны в антропогенно загрязненных ландшафтах и в чистых водоемах. Этот метод позволяет количественно оценить степень биологического воздействия загрязнений.

Ключевые слова: амфибия, печень, антропогенное загрязнение, мониторинг.

Introduction

The main difficulty in assessing the anthropogenically modified landscapes is the variety and unpredictability of acting factors, many of which can provide synergies. Therefore, methods to assess the cumulative physiological effects of anthropogenic contaminants directly on the organism of vertebrates, including humans, are particularly important. From this viewpoint, studies in animals for monitoring antropogenic pollution have no satisfactory alternatives. The green frogs klepton (*Pelophylax* kl. *esculentus*) is in many ways a favorable target for such studies (Boily et. al., 2005; Hopkins, 2007; Akulenko, Nekrasova, 2008). It is a complex of the synanthropic species with extensive habitat areal that covers a large part of Eurasia. Green frogs are found in freshwater ponds of any size, both clean and with a high degree of anthropogenic pollution. It is a complex species of amphibians, leading predominantly aquatic way of life, so it can be used for monitoring water pollution.

However, to assess the degree of contamination of the environment an essential technique for determining the physiological state of the animal from this environment is needed. The liver is the organ responsible for the body detoxification. It is most sensitive to the anthropogenic pollution. Studies on liver of the frogs caught in anthropogenically modified condition showed significant biochemical differences compared to the animals of cleaner habitats (Fenoglio et al., 2005; Falushinnska et al., 2008). Histological changes in the liver of amphibians are underinvestigated. However, the histological changes of the liver parenchyma are well studied in mammals. They are much easier to be interpreted in terms of "the norm — adaptive changes — pathological changes" than the deviation of biochemical parameters. Therefore, the aim of this study was to determine the histological changes in the green frog liver from anthropogenically modified landscapes and to compare them to those in the animals from pure landscapes. Based on these data, we propose a method for quantifying the degree of cumulative effect of the pollution on amphibia populations.

Material and methods

Animals (males and females *Pelophylax* kl. *esculentus*, body length of 5–12 cm, weight 12–45 g) were caught in agrocenosis around the village of Erchiki, in Kiev (within urban area and in green belt), in fisheries in the village of Zdorovka, on makeshift dump in the woods near the highway (with in the neighborhood of V. Alexandrovka). For a comparative study we involved the material collected in small lakes in the floodplain of the Desna (control of the cleanest places).

Liver preparations were stained with hematoxylin-eosin and Mallory. Histological changes were considered as a quality characteristics on the basis of the presence-absence. Statistical processing was performed by the standard method adopted for alternative characters, which allows you to find errors and the accuracy of the average difference between the samples of t-test (Lakin, 1973).

Results and discussion

Analysis of the results shows violations of the normal histostructure, which are characteristic of the mammalian liver under chronic toxicity condition. These include inflammation, foci of necrosis, fatty degeneration of hepatocytes, blood clots in large vessels (fig. 1, *A, B, D*). These changes indicate significant contamination of the investigated habitats, except the control and damp near the V. Alexandrovka (table 1). Loumbordis (2007) observed in the frog liver from polluted habitats, in addition to inflammation and "vacuolated focus" (apparently, it refers to fatty degeneration),

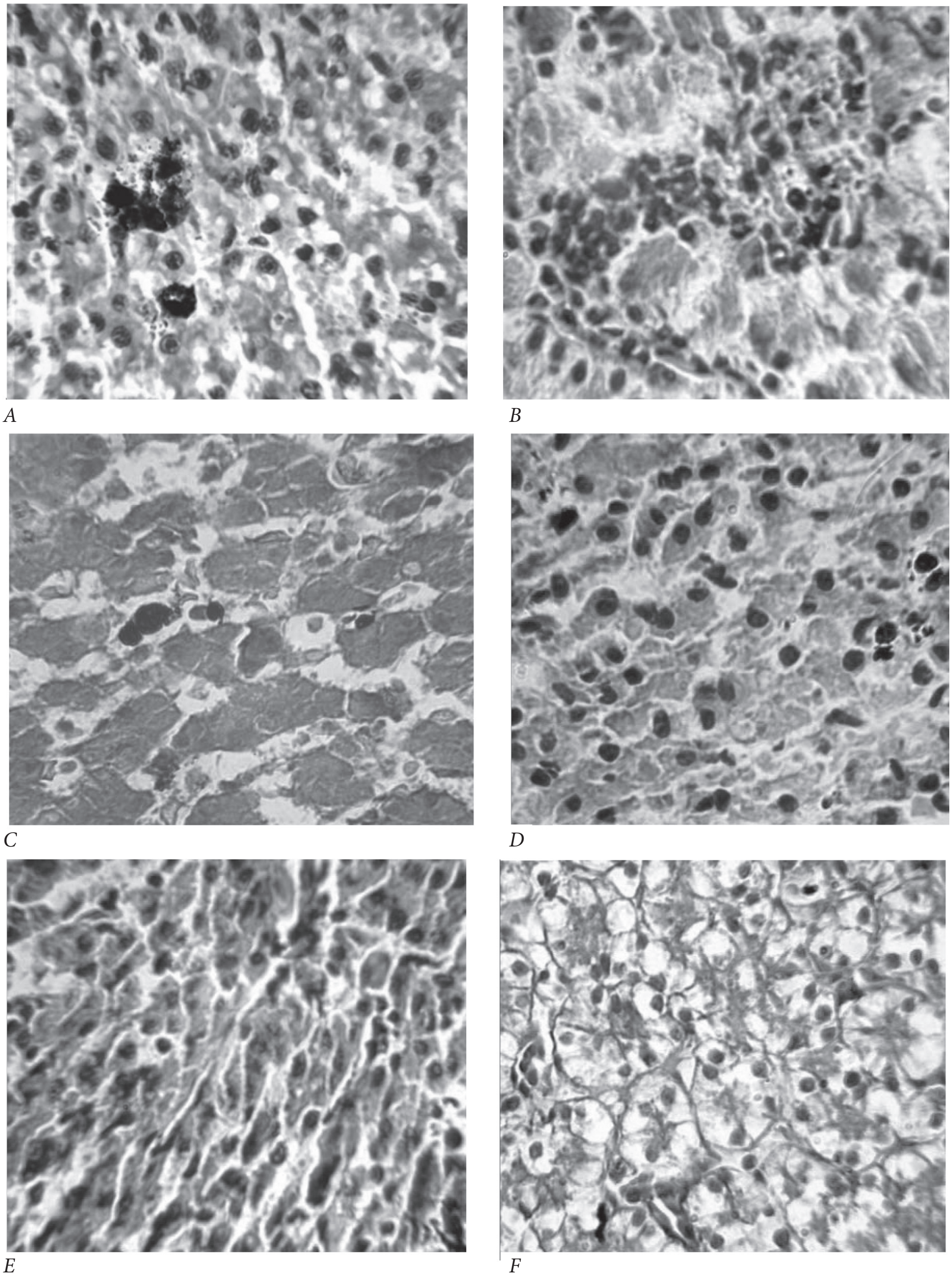


Fig.1. Alteration in the liver of the frog collected in the urban sprawl and in agrocenosis: *A* — fatty degeneration turning into necrosis; *B* — inflammation; *C* — degradation of the connective tissue; *D* — necrosis and the beginning of the anisocytosis in "emergency regeneration" (the bright pink cells with large nuclei); *E* — anisocytosis and abundance of small hepatocytes not forming a typical structure; *F* — protein dystrophy. Staining: hematoxylin-eosin (*A, B, D, E, F*) and Mallory (*C*). ×200.

Table 1. The presence of histological changes in the liver of green frogs from anthropogenic biocenosis with the different character of pollution (all in %) and the significance of differences with the control

Indicators	Control	Agrocenosis	Urban Sprawl	Dump	Fisheries	Forested Area
N	9	27	16	8	9	9
Inflammation	22 ± 13	3 ± 9	88 ±11 $p < 0.001$	29 ± 17	63 ±17 $p < 0.05$	100 ± 0 $p < 0.001$
Necrosis	0	81 ± 8 $P < 0.01$	88 + 11 $p < 0.001$	29 ± 17	100 ± 0 $p < 0.001$	100 ± 0 $p < 0.001$
Fatty degeneration	0	11 ± 6	30 ± 14	0	12.5 ± 11	12.5 ± 11
Protein dystrophy	0	68 ± 9 $p < 0.001$	0	0	0	0
Degradation of the connective tissue	0	59 ± 9 $p < 0.001$	100 ± 0 $p < 0.001$	14 ± 13	50 ± 17 $p < 0.05$	50 ± 17 $p < 0.05$
Anisocytosis	0	26 ± 8 $p < 0.01$	100 ± 0 $p < 0.001$	0	100 ± 0 $p < 0.001$	100 ± 0 $p < 0.001$
Presence of bands of small hepatocytes	0	19 ± 8 $p < 0.05$	70 ± 14 $p < 0.001$	0	25 ± 15	25 ± 15

the presence of fibrous structures of several types. However, according to our data, increasing of connective tissue around the vessels was identified in the liver lizards from polluted habitats, but not in the frog liver (Akulenko, 2006). In the study of frogs from agrocenosis, the protein degeneration of hepatocytes was identified as a specific change which is typical for the actions of a number of herbicides (Zhydenko, 2008). The protein dystrophy is often combined with hydropic. Arrays of cells with optically empty cytoplasm which are filled with separately lying protein granules, often are observed in the areas of damaged cells (fig. 1, *F*). Signs of the protein dystrophy were not detected in the liver of frogs from pure biocenosis or other anthropogenically modified landscapes. Liver damage is not accidental factor for the environmental pollution. Exposure to toxic substances directly causes damage of the hepatocytes. A direct causal link allows use not numerous samples for environmental monitoring. The absence of necrosis and fatty degeneration and a small percentage of inflammation in the samples of the liver is a proof of the biotope purity. In contrast, the presence of necrosis and fatty degeneration of the liver in animals from recreational area and fisheries indicates a significant pollution by motor transport.

Histological changes of liver parenchyma, which indicate the phenomenon of the so-called "emergency regeneration" were described by Akulenko (Akulenko, 2009). This is a characteristic of amphibians and it allows quick restoring the structure and function of the liver after severe necrotic changes. Under the condition of emergency regeneration the collagen layer between hepatocytes became thin; hepatocytes became smaller than normal and have an elongated shape. They do not form the typical girder structure (fig. 1, *C, E*). Also anisocytosis with the advent of dual-core cells is observed. The result of "emergency regeneration" is vast tracts of small hepatocytes which are not separated by layers of collagen.

In studies on newt after exposure to nonylphenol ethoxylates Bernabò et al. (2014) received largely similar results. In addition to displays of necrosis and the formation of fat droplets (fatty degeneration), the increase of intercellular spaces was named as the characteristic signs of toxicity. The presence of this feature is typical for the pattern of "emergency regeneration". The authors concluded that a short-term exposure to NPEs more negatively

affected the amphibian liver. This confirms that prolonged exposure triggers off the mechanism of "emergency regeneration."

From these data it may seem that the frogs from urban areas are less susceptible to necrosis of the liver than the animals of the cleaner places. In fact, in frogs from the city much of the foci of necrosis secondary substituted the zones of "emergency regeneration," which can take up to one third of the area of the cut. In frogs from agricultural landscapes foci of necrosis are usually not replaced by accelerated regeneration zones. One would assume that the liver necrosis and fatty degeneration of hepatocytes in agrotcenoze like in the urban landscape, is caused by oil pollution associated with road transport (Akulenko, 2006). However, the areas of protein dystrophy often move into areas of cells destruction or necrosis. Therefore the cause of hepatocyte necrosis can be an immediate pollution by pesticides. On the other hand, in the frogs from agrocenosis hepatocyte anisocytosis is found much less frequently, which is a direct indication of the process of liver regeneration (Karapetyan, Jivanyan, 2006). Therefore, we can assume that the possibility of liver regeneration by the action of pesticides and fertilizers are significantly reduced.

As seen from the table, the quantitative analysis can identify significant differences in the pattern of pathological changes in the liver of the green frogs collected in anthropogenically contaminated landscapes, compared to the control. These differences can be safely attributed to the toxic substances of human origin. These results also show that the contaminated biocenosis demonstrates significant differences from the control in the analysis of very small samples (less than 10 animals). This allows us to assess the status of water bodies with small populations of frogs, which are characteristic of anthropogenically modified landscapes.

Conclusion

Histological examination of the liver of frogs caught in anthropogenic landscapes allows to determine the presence of contamination and, to a certain extent, to reveal its nature and gravity. This method is promising for further development in order to monitor anthropogenic pollution.

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